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AUTHORITY

AMXFD ltr, 9 Feb 1972

THE LUMINESCENCE OF LUMINOL IX

- Yugoslavia -

/Following is a translation of an article by K. Weber, Lj. Huic and M. Mrazovic, of the Institute for Medical Research of the Yugoslav Academy of Sciences and Arts, Zagreb, in the Croatian-language periodical Arhiv higienskoga rada (Archives of Hygiene), No. 9, Zagreb, 1958, pages 325-347./

The Catalytic Action of Isopestox on the Chemiluminescence of Luminol and the Inhibition of this Reaction /See Note/

/Note:/ Eighth paper of this series: K. Weber and R. Kostelac, Croat. Chem. Acta 28 (1956) 33.

Quantitative photoelectric measurements of the intensity of emitted light were used to investigate the catalytic action of isopestox on the chemiluminescence of luminol in the presence of hydrogen peroxide in alkaline solutions. Isopestox in relatively small concentrations considerably increases the intensity of the luminescence of luminol. The Michaelis constant was determined for this reaction, which may be regarded as a model reaction of the enzymatic (peroxidative) effect of the organophosphoric compound isopestox.

Various inorganic or organic substances act as effectors on the chemiluminescence of luminol catalyzed by the addition of isopestox.

The effector action of foreign substances (inorganic salts, polyphenols and aromatic amines) is mostly manifested as inhibition (extinction of the luminescence). However, there are also substances which considerably raise the intensity of luminescence, or which increase the strength of luminescence of luminol in reduced concentrations, but decrease it in larger concentrations.

It is significant that isopestox, which, as an organophosphoric compound, possesses the faculty of inhibiting enzymatic reactions, acts in this concrete case in vitro exactly like the enzyme peroxidase, in which case the main laws of the kinetics of enzymatic reactions operate. But the laws of the inhibition of enzymatic reactions are not confirmed when the inhibition of this reaction is tested by adding foreign substances. It appears that the mechanism of the luminol reaction is so involved that the foreign substances (effectors) in the reaction mixture may affect the course of the reaction in various directions.

The catalytic effect of isopestox on the luminol reaction may serve for the quantitative determination of this substance by means of photoelectric measurements of the intensity of luminescence.

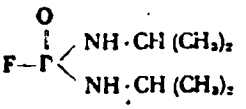
It is known that organophosphoric compounds act catalytically on oxidation reactions taking place under the influence of hydrogen peroxide /1/ /Numbers in brackets refer to similarly numbered items in Bibliography at end./ The organophosphoric compound in such reactions has the role of transferring the active oxygen of the hydrogen peroxide to some substrate, which is oxidized, and therefore it may be considered that this phosphorus compound acts fundamentally in the same way as the enzyme peroxidase. The substrates of these model reactions for the peroxidative action of organophosphoric compounds may be of various sorts, and intensely colored substances /2/, compounds possessing the property of fluorescence /3/, etc., may be formed as the products of oxidation. It has recently been ascertained that certain very poisonous organophosphoric compounds (nerve poisons) intensively catalyze the chemiluminescence of luminol in the presence of sodium perborate as donor of active oxygen /4/. In the presence of minimal quantities of strong nerve poisons,

the intensity of the chemiluminescence of luminol increases considerably, and hence this reaction has been proposed for the detection of such poisons. The catalyzed reaction of luminol is, in fact, also a model reaction for peroxidative effect, and sodium perborate as the reactive component, $NaBO_3 \cdot 4H_2O$, to which the "constitutional formula"

$$NaBO_2 \cdot H_2O_2 \cdot 3H_2O,$$

actually corresponds better, serves as the source of hydrogen peroxide. In addition, perborate gives the required alkaline reaction to the solution.

In connection with these known facts, it was of interest to ascertain how other less poisonous organophosphoric compounds, of the insecticide group, act on the luminol reaction and what are the kinetic properties of these actions. Since it has been found through qualitative tests of a large number of organophosphoric insecticides that isopestox (Mipafox, bis-monoisopropylamino-fluoro-phosphinoxyde)



intensively catalyzes the luminol reaction in alkaline solutions in the presence of hydrogen peroxide, the more detailed investigation of this catalytic effect was approached particularly from the viewpoint of the kinetics of enzymatic reactions.

Work Method

Experiments with the action of isopestox on the chemiluminescence of luminol were performed with alkaline solutions of luminol (3-aminophthalhydrazide) in the presence of hydrogen peroxide. The concentration of luminol was varied in the reaction mixture, the Michaelis constant being determined within the limits of 0.8×10^{-4} M and 16×10^{-4} M. The experiments with inhibition were always performed with a luminol concentration of 8×10^{-4} M in the reaction solution. The alkali concentration in these mixtures was always 9×10^{-2} M of NaOH, and the hydrogen-peroxide concentration 2.52×10^{-2} M. The isopestox concentration was varied within the limits of 2.75×10^{-3} M and 11.0×10^{-3} M; in the tests for inhibition it was always 5.5×10^{-3} M. The total volume of

the reaction mixture was always 50 ml.

The intensity of the chemiluminescence was measured with photoelectric apparatus with a seleniun photoelement and a lightable mirror galvanometer, described in earlier papers /5/. The inclination of the galvanometer (G) served as a relative measure of the luminescence intensity at the moment of measurement. The chemiluminescence intensity varies in the course of the reaction; at the beginning of the reaction it rises quickly to a certain maximum, while in the further course of the reaction it diminishes gradually, and at the end the luminescence is entirely extinguished. The whole course of the reaction was accompanied by readings of the galvanometer inclination at intervals of five seconds. The isopestox solution was added to the mixture of solutions of the other reaction components immediately before the beginning of the work, and at the end of five seconds the first galvanometer-inclination reading was taken. The maximum inclination of the galvanometer (Gm) in these experiments represents the relative measure for the speed of the reaction of luminol with hydrogen peroxide, or the relative measure for action of isopestox on the luminol reaction under the stated experimental conditions. The reaction of luminol with hydrogen peroxide without the presence of a catalyst is so slow that its speed may be disregarded in comparison with that of a catalyzed reaction in kinetic considerations.

Results of the Work

To investigate how isopestox acts on the luminol reaction, this reaction was regarded as a model enzymatic reaction, and was treated by the kinetic method as propounded by L. Michaelis and M. Menten /6/ and others /7, 8/. According to the theory of these authors the speed of enzymatic reactions (V) is best characterized by the equation:

$$V = \frac{Vm \cdot [S]}{Ks + [S]}. \qquad (1)$$

in which Vm signifies the maximum speed (speed with a very high concentration of substrate), /S/ the substrate (luminol) concentration, and Ks the Michaelis constant. This constants corresponds to the dissociation constant of the compound of substrate and enzyme, in this concrete case the compound of the peroxide, luminol and isopestox /9/.

The constant Ks was determined for the chemiluminescence of luminol in the present of isopestox by measurements

of the luminescence intensity with different concentrations of luminol and with a constant concentration of isopestox. One series of such luminescence curves obtained is shown in Figure 1. In this figure, G signifies the relative strength of luminescence (inclination of the galvanometer of the photoelectric apparatus). and t the time of reaction in seconds. The luminol concentration was varied within the limits of 0.8×10^{-4} M and 16×10^{-4} M, and the isopestox concentration was always 1.1×10^{-3} M. It may be seen that the maximum strength of luminescence (Gm), as well as the total light (integral) of the curves in Figure 1) grow considerably with the growth in the substrate concentration, and this corresponds to equation (1).

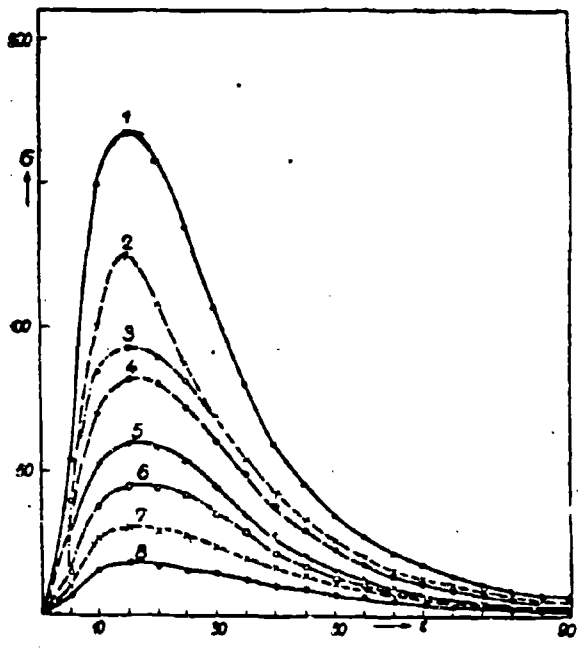


Figure 1. Curves of the intensity of chemiluminescence as a function of the reaction time with different luminol concentrations.

Curve 1: 16×10^{-4}, curve 2: 12×10^{-4}, curve 3: 8×10^{-4}, curve 4: 6.4×10^{-4}, curve 5: 4.8×10^{-4}, curve 6: 3.2×10^{-4}, curve 7: 1.6×10^{-4}, and curve 8; 0.8×10^{-4} M of luminol. Isopestox concentratio.

1.1 x 10^{-3}. G = inclination of the galvanometer (relative measure for intensity of luminescence), t = time of reaction in seconds.

Similar series of experiments were also performed for two other constant concentrations of isopestox, namely 5.5 x 10^{-4} M and 2.2 x 10^{-3} M. The maximum luminescence intensities obtained are shown in dependence upon the luminol concentration (c) in Figure 2. It may be seen that Gm changes regularly with the growth of the concentration of luminol as well as isopestox. The numerical value of Ks was obtained from these data graphically by M. Dixon's procedure (10). By transformation of equation (1) one obtains:

$$\frac{1}{V} = \frac{Ks}{Vm} \cdot \frac{1}{[S]} + \frac{1}{Vm} \qquad (2)$$

and it may be seen that the graph of 1/V as a function of $1/\sqrt{S}$ yields for various concentrations of isopestox (different Vm) directions which must intersect the abscissa at the same point. This point on the abscissa corresponds to the reciprocal value of the Michaelis constant (-1/Ks). For the luminol reaction with isopestox as a catalyst, the directions were obtained for the above-mentioned concentrations of the catalyst which are shown in Figure 3. For -1/Ks a value of 4.5 x 10^{-3} was obtained, and for the Michaelis constant

$$Ks = 2.2 \cdot 10^{-4}.$$

From the above results it is clear that the usual kinetic treatment of enzymatic reactions may be applied in principle to the chemiluminescence of luminol catalyzed with isopestox.

In another part of this study an investigation was made of the effector action of various foreign substances on the chemiluminescnece of luminol catalyzed with isopestox. Also used as foreign substances were both inorganic salts and organic compounds, predominantly phenols and aromatic amines. Substances were chosen which are known to be effectors of the luminol reaction in the presence of other catalysts /11/.

Potassium iodide acts only as a pronounced inhibitor of the luminol reaction. Already in very small concentrations it diminishes the maximum strength (Gm) of luminescence and the total light, i.e., the aggregate energy of emitted light.

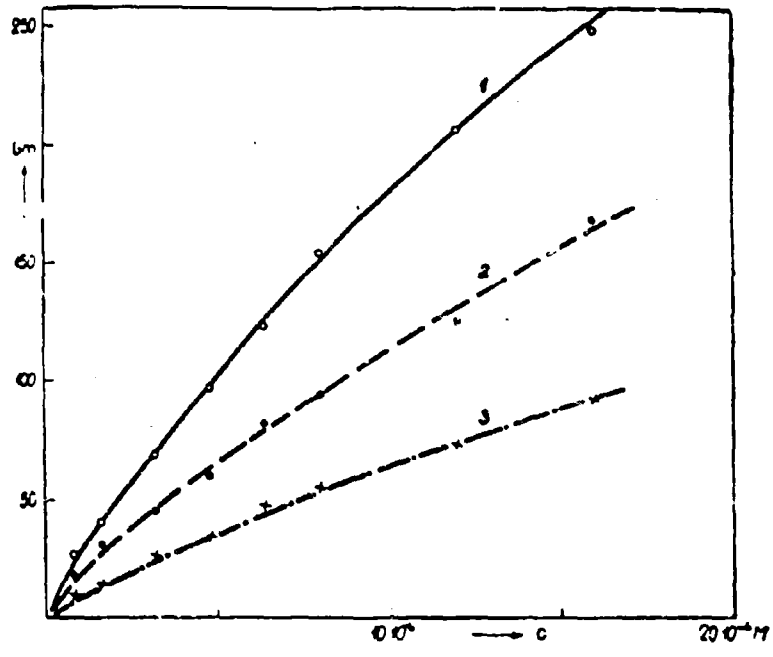


Figure 2. Dependence of the maximum intensity of luminescence (GM) on the luminol concentration (c) with different isopestox concentrations.

Curve 1: 2.2×10^{-3}, curve 2: 1.1×10^{-3} and curve 3: 5.5×10^{-4} M of isopestox.

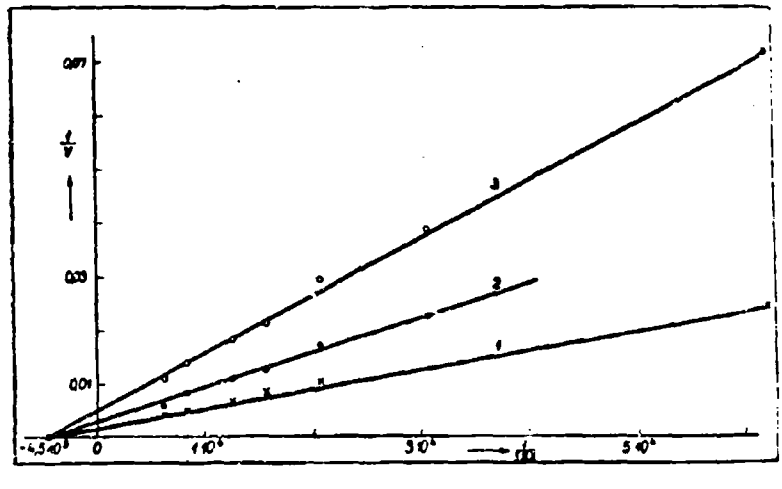


Figure 3. Graph of the results according to

equation (2). V = speed of reaction (maximum strength of luminescence); S = concentration of the substrate (luminol). Isopestox concentration as in Figure 2.

Figure 4 shows one series of chemiluminescence curves obtained from the addition of potassium iodide in different concentrations. It may be seen that the potassium iodide in a 0.2 M concentration almost completely extinguishes the luminescence. Figure 5 shows graphically the dependence of the

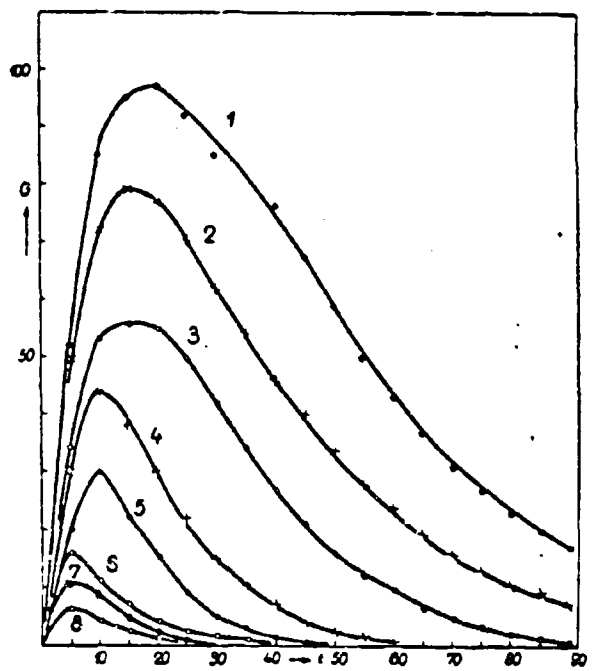


Figure 4. Curves of chemiluminescence in the presence of different concentrations of potassium iodide.

Curve 1 without KI, curve 2 0.001, curve 3 0.004, curve 4 0.010, curve 5 0.020, curve 6 0.060, curve 7 0.100 and curve 8 0.200 M KI. G = relative strength of luminescence. t = time of reaction in seconds.

maximum strength of luminescence on the concentration of potassium iodide. A halved concentration of this inhibition, i.e. the concentration of potassium iodide which reduces the strength of luminescence to one half (50%), amounts to:

$$c_{1/2} = 8{,}2 \cdot 10^{-3}\ M,$$

and since the experiments were performed with an isopestox (catalyst) concentration of 5.5×10^{-3} M, this means that nearly every inhibitor ion can impede the action of a molecule of the catalyst. Upon mathematical treatment of the results obtained, it was found that the general inhibitor equation

$$\frac{v_0}{v} = 1 + \beta \cdot c \qquad (3)$$

(v_0 and v are the speeds of reaction without the presence of an inhibitor, or with an inhibitor concentration c, and β is the inhibitor constant) is only approximately valid for the action of the iodide on chemiluminescence. With potassium-iodide concentrations higher than 0.02 M, the inhibitor constant decreases considerably with the growth in this concentration. This irregular decrease in the inhibitor effect is

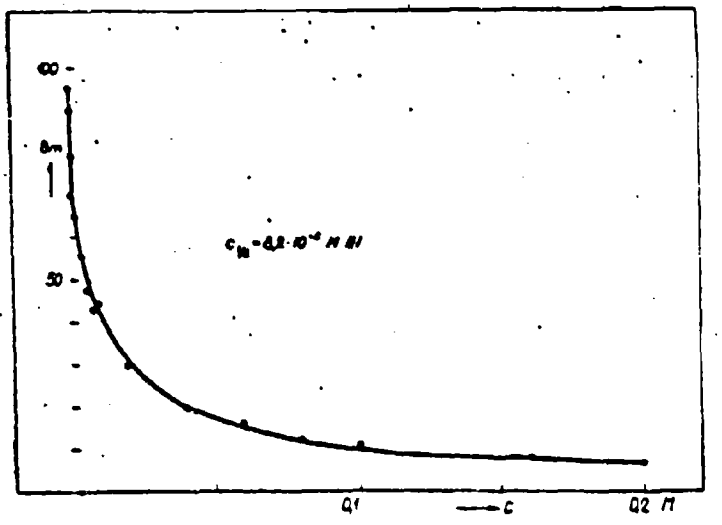


Figure 5. Dependence of the maximum strength of luminescence (Gm) on the concentration of potassium iodide (c).

felt still more with smaller iodide concentrations if the experiments are performed with a smaller luminol concentration (4.0×10^{-4} M).

Potassium rhodanide = "thiocyanate", the more generally accepted name, which, along with iodide, is known as a good inhibitor of other reactions, acts as a pronouncedly positive effector on this chemiluminescence. With small concentrations it considerably increases the maximum strength of luminescence, while the total light diminishes somewhat. When the rhodanide concentration is more considerably increased, the total light also increases along with the maximum strength of luminescence. Finally, at a rhodanide concentration above 0.05 M, the maximum strength of luminescence again diminishes, while the total light continues to increase. Figure 6 shows a series of chemiluminescence curves in the presence of various concentration of potassium rhodanide, and figure 7 gives the dependence of the maximum strength of luminescence on the rhodanide concentration. It may be seen that the reaction mixture always glows more strongly in the presence of rhodanide than without it, and the maximum strength

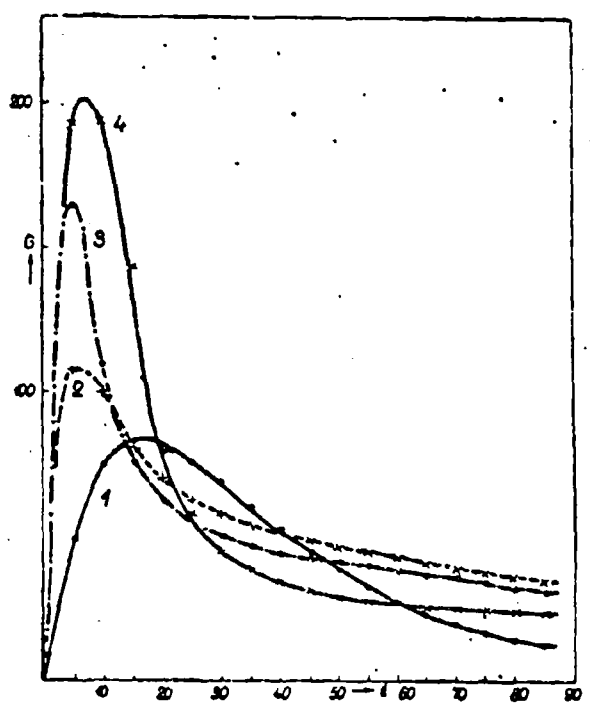


Figure 6. Curves of chemiluminescence in the presence of different concentrations

of potassium rhodanide.

Curve 1 without KCNS, curve 2 0.200, curve 3 0.100 and curve 4 0.040 M KCNS. G = relative strength of luminescence, t = time of reaction.

of luminescence increases to double the value at a rhodanide concentration of

$$c_1 = 0.022 \text{ M}.$$

Since the curve in Figure 7 shows the maximum, while the total light at first diminishes somewhat in dependence upon the rhodanide concentration, but then gradually increases with the further growth of the concentration, it is evident that the rhodanide really acts in two directions, namely both as a positive catalyst and as an inhibitor, with the positively catalytic effect predominating. As a result of this double action, a reduced positive effector action is obtained with an optimum concentration at 0.048 M potassium rhodanide.

The effector action of potassium bromide consists in a considerable increase in the maximum strength and total

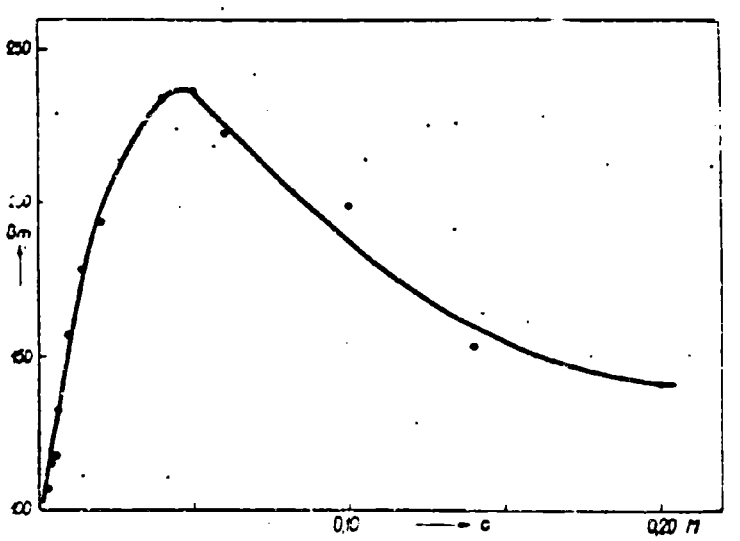


Figure 7. Dependence of the maximum strength of luminescence (Gm) on the concentration of potassium rhodanide (c).

light of the luminescence of luminol in the presence of isopestox. Figure 8 shows a series of curves of luminescence in the presence of growing potassium bromide concentration, and Figure 9 gives the dependence of the maximum strength of luminescence upon the potassium bromide concentration. From these graphs it may be ascertained that potassium bromide in a 0.2 M concentration in the reaction mixture increases the total light of luminescence in the proportion of 1:6.92 and the maximum strength of luminescence in the proportion of 1:5.96. The bromide concentration which doubles the maximum strength is

$$c_{\frac{1}{2}} = 0{,}025\ M.$$

It might perhaps be thought that the effector action of potassium bromide, and of rhodanide as well, is a form of

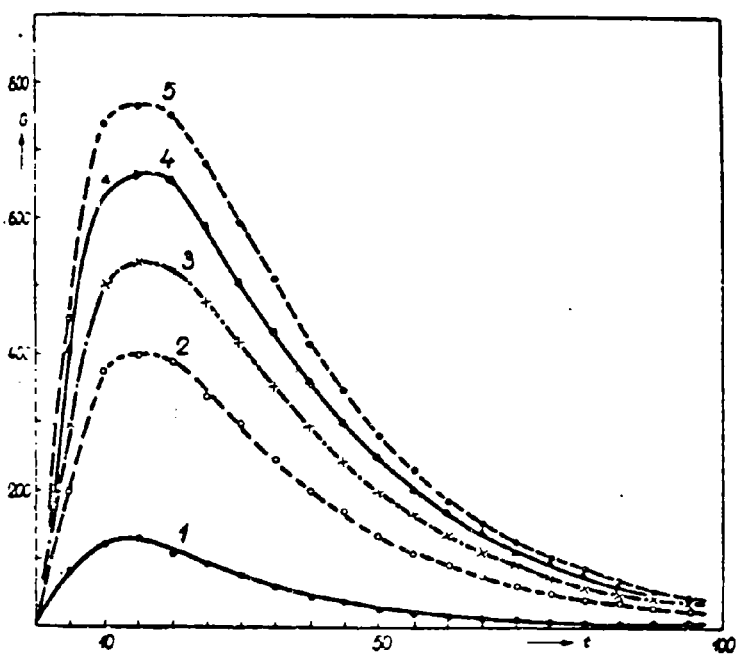


Figure 8. Curves of chemiluminescence in the presence of different concentrations of potassium bromide.

Curve 1 without KBr, curve 2 0.04, curve 3 0.10, curve 4 0.14 and curve 5 0.20 M KBr. G= relative strength of luminescence, t= time of reaction.

the primary electrolytic effect in the sense of the Bronsted theory [12]. This assumption has, however, become improbable, as it has been found that other electrolytes, such as potassium chloride and nitrate, do not act as effectors on this reaction.

Among the organic compounds, investigation was made into the effector action of amines and phenols on the chemiluminescence of luminol in the presence of isopestox, as shown in Table 1. Apart from hydroquinone and phenol, the substances listed there act only as inhibitors, and the halved concentrations of this inhibitory action ($c_{\frac{1}{2}}$) are given in the table. Methol, pyragallel and resorcin are very active inhibitors, since their halved concentrations of inhibition are smaller than the concentration of luminol substrate. For methol $c_{\frac{1}{2}}$, the proportion to the luminol concentration is even 0.00825:1, i.e. a halved inhibitor concentration about 100 times smaller than the substrate concentration. All the inhibitors listed also lower the maximum strength of luminescence and the total light, but not equally.

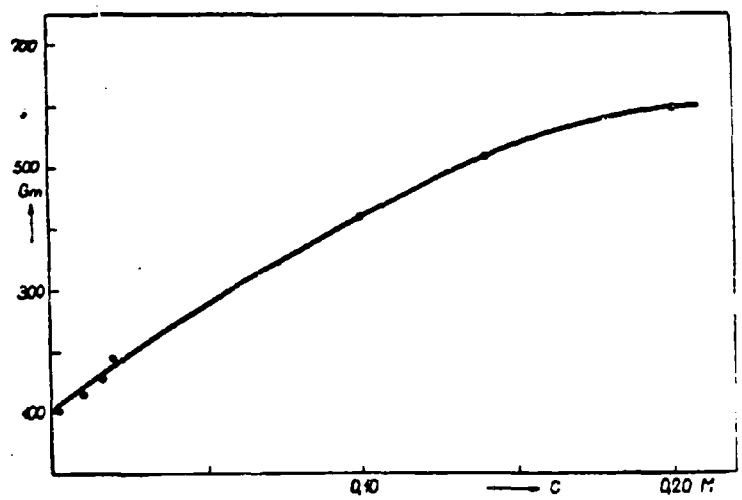


Figure 9. Dependence of the maximum strength of luminescence (Gm) on the concentration of potassium bromide (c).

Table 1

Halved inhibitor concentrations $c_{\frac{1}{2}}$

Inhibitor	$c_{\frac{1}{2}}$ M	Inhibitor	$c_{\frac{1}{2}}$ M
Aniline	3.5×10^{-3}	Methcl	6.6×10^{-6}
Phenol	5.4×10^{-2}	Pyrogallol	1.1×10^{-4}
Hydroquinone	3.5×10^{-4}	Ascorbic acid	3.3×10^{-4}
Resorcin	4.8×10^{-4}	Isopropanol	6.5×10^{-1}

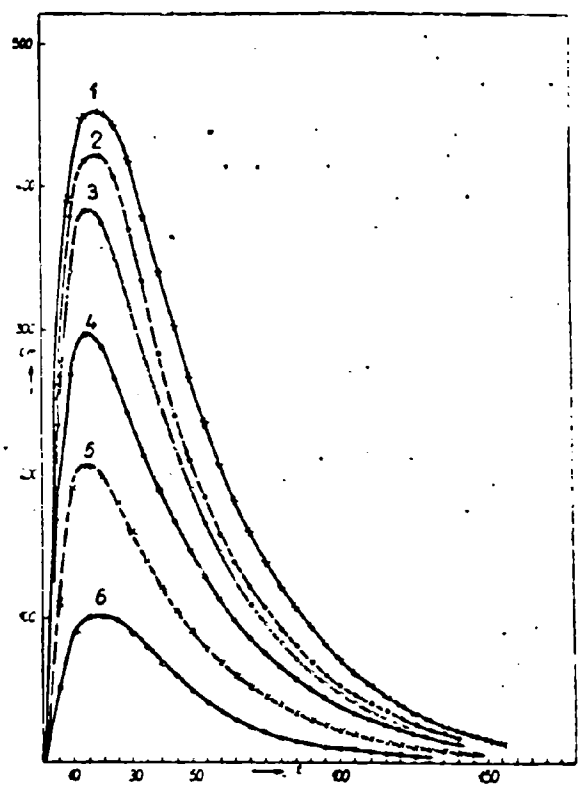


Figure 10. Curves of chemiluminescence in the presence of phenol.

Curve 1 without phenol, curve 2 0.002, curve 3, 0.004, curve 4 0.006, curve 5 0.008 and curve 6 0.014 M of phenol. G=relative strength of luminescence, t= time of relation.

Hydroquinone and phenol act in two directions on the chemiluminescence of luminol catalyzed by i opestox: as a positive effector in smaller concentrations, and as an inhibitor in larger ones. Figure 10 shows the luminescence curves with positive effector action of phenol, and Figures 11 and 12 the dependence of the maximum strength of luminescence on the concentration of phenol and hydroquinone. It may be seen that hydroquinone acts only weakly as a positive effector but strongly as an inhibitor, whereas phenol acts very prenouncedly in both directions. It is significant that addition of phenol in a 0.014 M concentration causes the maximum strength of the luminescence to increase in the proportion of 1:4.44, and the total light in the proportion of 1:5.42. Accordingly, the emission of luminescence light can indeed be considerably increased by such an effector.

Since the luminol reaction involves an oxidation process, namely the dehydration of the luminol, an investigation was made of the effect of a pronounced reducing agent, ascorbic acid, on chemiluminescence. It was found that ascorbic acid strongly inhibits the luminol reaction catalyzed by isopestox (see Table 1), while both the maximum strength of luminescence and the total light diminish about equally.

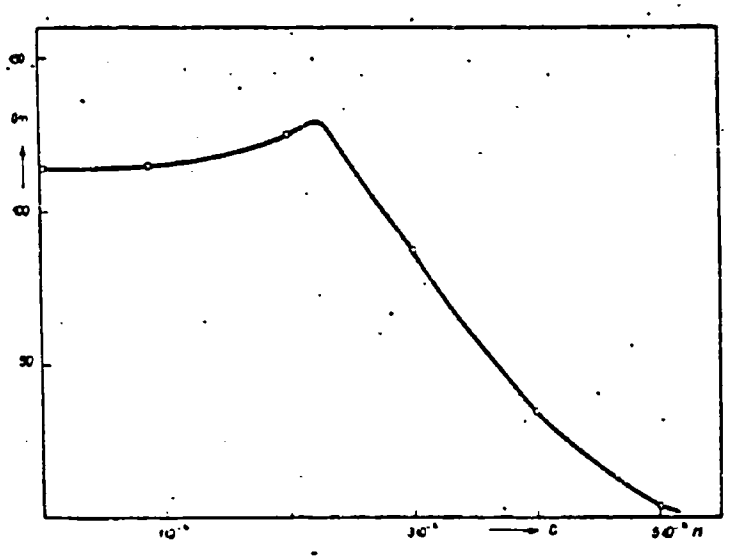


Figure 11. Dependence of the maximum strength of luminescence (Gm) on the concentration of hydroquinone (c).

Isopestox dissolves easily in water, and the above-described experiments were made with aqueous solutions. However, most of the other organophosphoric compounds do not dissolve in water, and it is customary to work with them in isopropanol solutions. Hence it was of interest to ascertain how isopropyl alcohol acts on the luminescence of luminol catalyzed by isopestox. The experiments made in this direction have shown that isopropanol weakly inhibits this reaction. The halved inhibitor concentration is considerably larger for this substance than for the other inhibitors investigated (see Table 1). It may be considered that in this case it is really not a matter of true inhibition, but probably a solvent effect /13/.

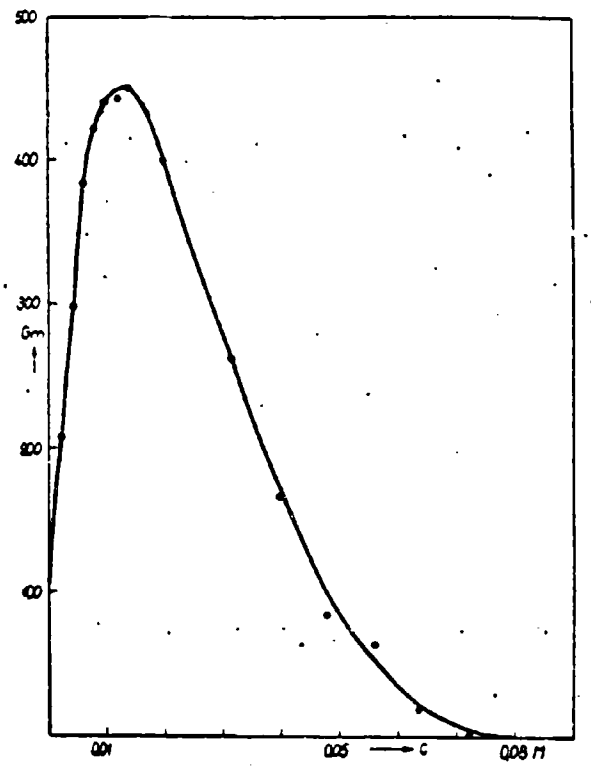
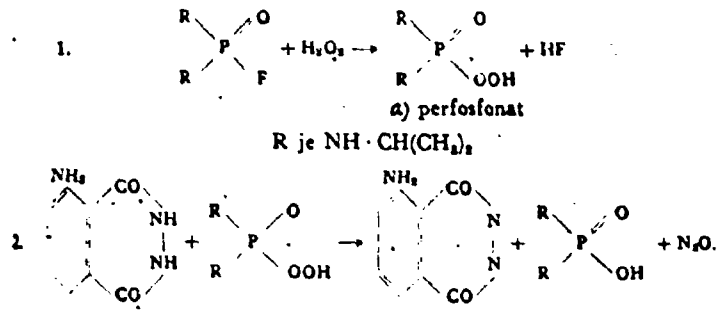


Figure 12. Dependence of the maximum strength of luminescence (Gm) on the concentration of phenol (c).

Discussion of the Results

It may be considered that the reaction of isopestox with luminol and hydrogen peroxide in alkaline solutions takes places in fundamentally the same way as the Schonemann reaction [14]. A rough diagram of the reaction would accordingly be:



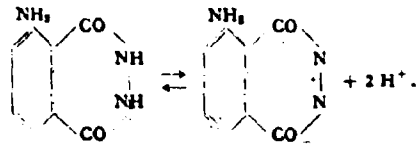
[Legend:] a) perphosphonate.

The first of these reactions formed under the influence of hydrogen peroxide on the isopestox is perphosphonate, which contains an unstably bound oxygen atom. In alkaline solutions, the hydrogen-peroxide anion formed by dissociation

$$H_2O_2 \rightleftarrows H^+ + OOH^-.$$

reacts with the isopestox.

In alkaline solutions, moreover, luminol anions are formed in equilibrium :



so that the perphosphonate reacts with these anions. Evidently the luminol anions form with the perphosphonate an unstable complex which breaks up into dehydrated luminol and diakylphosphinic acid. The energy released in this breakup serves to excite the luminol molecule or ion, and this energy

is finally emitted in the form of luminescence light. The last phase of the luminol reaction is certainly governed by a very complicated reaction mechanism. Since according to the above diagram every luminol molecule is dehydrated by reaction with one isopestox molecule, and this "catalyst" is not regenerated during the reaction, but it is converted into dialkylphosphinic acid, which cannot activate the oxygen of the hydrogen peroxide, the isopestox really does not play the role of catalyst, but that of a reaction component. This assumption is further confirmed by the fact that efficacious chemiluminescence manifests itself only in the presence of isopestox in a concentration equal in order of magnitude to the luminol concentration.

For the above reaction diagram it is significant that J. Epstein and collaborators /15/ found that hydrogen peroxide can speed up the basic hydrolysis of some organophosphoric compounds, with the development of elementary oxygen (catalytic action of the organophosphoric compound). Such processes might also take place in the luminol reaction in the presence of large concentrations of hydrogen peroxide and small concentrations of luminol. But in the normal luminol reaction, the above-mentioned reactions, corresponding to the peroxidative action of isopestox, take place first.

In the formal kinetic treatment of the results obtained it was considered that the maximum strength of luminescence (Gm) also corresponds to the maximum speed of the luminol reaction. Since the maximum strength of luminescence is usually reached in about 15 seconds of the reaction time (see Figure 1), it is clear that the luminol reaction has, from the formal kinetic standpoint, the character of an autocatalytic reaction. It is well to treat such reactions kinetically by examining precisely the maximum speed of reaction and by determining the influence of the various factors on this speed. Hence the maximum strength of luminescence was used in applying the Michaelis theory (6) to the luminol reaction as the relative numerical measure of the speed of the reaction. The results obtained from these calculations showed (see Figure 3) that this procedure was correct, and that the theory in question may be properly applied to the luminol reaction.

In the kinetic examination of the action of foreign substances (effectors) on the luminol reaction, the maximum strength of luminescence may be used also as a measure of the speed. It may, however, also be ascertained how the foreign substances act on the total quantity by the reaction of the converted substances. The total light of chemiluminescence

represents a relative measure of this quantity of chemically converted substances, in fact of the luminol, since the inhibitors can make possible other reaction ways of consuming the "catalyst" (isopestox) as well as the hydrogen peroxide, and the luminol does not enter into reaction with these substances. Kinetically there is no fundamental difference in the investigation of effector actions, if calculated on the basis of the maximum strength, or on the basis of the total light of luminescence.

The results obtained for the inhibitory action of the different substances on the chemiluminescence of luminol in the presence of isopestox were treated mathematically by the methods usual in work in the field of the inhibition of enzymatic reactions [7, 8, 10, 16]. It was found, however, that the usual laws of inhibition cannot, in this case, properly interpret the results obtained. For the dependence of v_o/v on the concentration of the inhibitor, a linear relationship was obtained only for the action of aniline on the luminescence. This means that the general inhibitor equation (3) is valid only for this case of inhibition. In the other cases listed in Table 1, v_o/v increases faster with the growth of the inhibitor concentration than according to equation (3). This means that the inhibition is considerably more pronounced at high concentrations of the inhibitor than we would expect in the case that equation (3) is valid. This statement is especially true for inhibition under the influence of progallol, methol and ascorbic acid, less so for inhibition by resorcin. Tables 2, 3, and 4 gove the values computed according to equation (3) for the constant β, namely for inhibition by aniline, or by pyrogallol and ascorbic acid. It may be seen that the β values for aniline are fairly constant, while for pyrogallol and ascorbic acid they rise rapidly with the increase in the inhibitor concentration.

Table 2

Inhibition by analine

Anilin M	V	Vo V	$\beta \cdot 10^{-3}$
—	100	1,00	—
0,002	65,2	1,53	2,15
0,006	33,7	2,96	3,27
0,010	25,6	3,91	2,91
0,014	19,6	5,11	2,94
0,020	17,0	5,75	2,37

Table 3

Inhibition by pyrogallol

Pirogalol M.10⁴	V	Vo V	$\beta \cdot 10^{-3}$
—	100	1,00	—
6	90,1	1,10	1,67
8	82,8	1,20	2,50
14	69,4	1,44	5,14
20	54,2	2,09	9,65
30	11,7	8,58	25,2
40	3,6	27,8	67,0

Table 4

Inhibition by ascorbic acid

a) Askorbinska kiselina M.10⁴	V	Vo/V	$\beta \cdot 10^{-3}$
—	100	1,00	—
1	90,4	1,11	1,05
3	54,5	1,84	2,80
4	41,0	2,44	3,05
5	19,3	5,18	8,35
6	8,4	11,9	18,1
8	0,6	11,6	19,4

/Legend:/ a) Ascorbic acid M x 10^4.

The above-mentioned fact that the gereral inhibitor equation (3) is not valid for most of the inhibitors used clearly shows that these inhibitions are of a complex nature. Evidently dual, or perhaps even triple, influences are involved. Hence there would not be any sense in trying to determine by the performance of the respective experiments what types of inhibition were involved in the concrete cases. In this connection attention should also be called to the fact that in chemiluminescence the inhibitors may act not only on the luminol reaction, which produces excited molecules (ions), but also on the excited (activated) molecules during the excitement. In this respect there is an essential difference between the usual enzymatic reactions and the luminol reaction.

Practical Application

Within the framework of this study, the results obtained on the action of isopestox on the chemiluminescence of luminol may also serve for the analytic determination of this organophosphoric compound, namely by measurements of the intensity of luminescence. For this purpose it is necessary to gauge the photoelectric apparatus with a series of different concentrations of isopestox with a constant concentration of the other components of the reaction and under other constant work conditions. Figure 13 shows the series of chemiluminescence curves thus obtained. It may be seen that both the maximum strength and the total light considerably increase with the growth of the isopestox concentration. Figure 14 gives the functional dependence of the

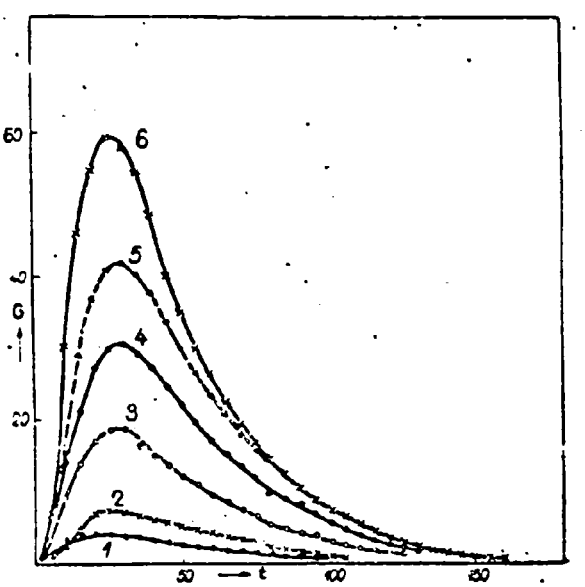


Figure 13. Curves of chemiluminescence in the presence of different concentrations of isopestox.

Curve 1 0.002, curve 2 0.004, curve 3 0.010, curve 4 0.020, curve 5 0.030 and curve 6 0.040% isopestox in the reaction mixture.

maximum strength as well as the total light of luminescence on the isopestox concentration. The maximum strength of the

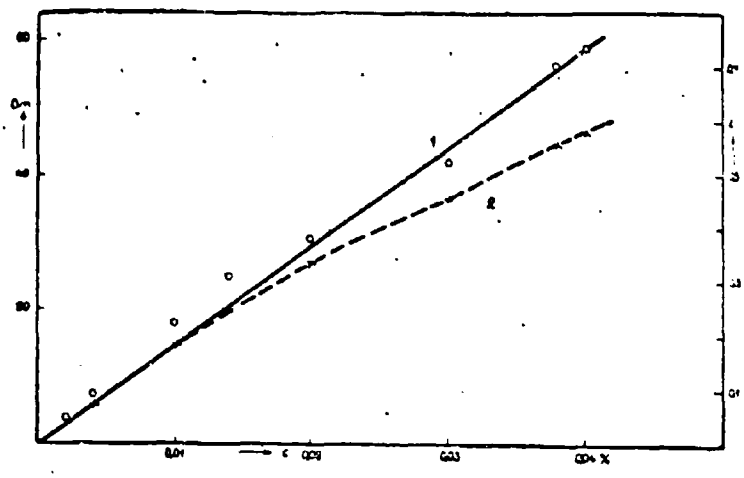


Figure 14. Dependence of the maximum strength (Gm, curve 1) and total light (L, curve 2) on the concentration of isopestox (c).

luminescence of luminol increases with the growth of the isopestox concentration, but the oscillations of the values around this linearity are fairly pronounced. The total light (L), on the contrary, does not increase at all linearly with the growth of the isopestox concentration, but the oscillations of the individual values around the normal flow of the curve are very insignificant. This means that the determinations of the total light are considerably more precise and certain than those of the maximum strength of luminescence. This fact is easy to understand if we take into consideration that the relative numerical value of the total light of luminescence represents the collective result of a large number of measurements (up to more than 30 galvanometer readings), whereas the maximum strength of luminescence is obtained by only one measurement of the inclination of the galvanometer.

In photoelectric determinations of isopestox, by employment of the chemiluminescence of luminol, two possible work methods are provided. The first determines by a single galvanometer reading the relative maximum strength of luminescence, while the other measure the whole time flow of the intensity of luminescence, and the total light is determined by graphic integration. In both cases one works on the basis of gauging curves, on which the concentration of isopestox in the solution studied is determined by graphic interpolation.

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